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Stable triterpenoid iminium salts and their activity as inhibitors of butyrylcholinesterase

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ABSTRACT

Amides derived from platanic acid (30-nor-lupane skeleton) were converted in two steps into 21-aza-17,19-ethano-19-nor-urs-20-en-21-ium chlorides. These ursane-skeleton iminium salts are first in their class, and some of them proved as selective inhibitors of the enzyme butyrylcholinesterase while holding no inhibitory activity for acetylcholinesterase.

1. Introduction

In the search for novel bioactive triterpenes, derivatives carrying - compared to the parent compound - one or more additional amino groups have been the focus of scientific attention for some time [1–18]. For example, the cytotoxic potential of pentacyclic 2-amino triterpene carboxylic acids has been known for some time but many of these compounds are equally cytotoxic to tumor cells and non-tumor cells. Higher selectivity (with concomitant high cytotoxicity) has been observed compounds bearing either distal amino groups, or aromatic amines of the quinoline or isoquinoline type [19], quaternary ammonium ions [20,21] and especially lipophilic cations such as triphenylphosphonium salts [22–29], mitochondria toxic cationic F16 [30,31] or rhodamine B derivatives [32–37]. Also of high and increased interest are those derivatives bearing additional fused rings with an additional nitrogen function. Triterpenoid azepanes appear [38–42] to be particularly noteworthy since they can also act as inhibitors for the enzymes acetyl- and butyrylcholinesterase (AChE, BChE). Inhibitors of these enzyme play an important role in the therapy of several neurodegenerative diseases. The latter class of compounds is usually obtained by reduction of the corresponding lactams, which in turn are readily accessible in high yields via Beckmann rearrangements from oximes [43,44].

2. Results and discussion

Recently, we discovered an “unusual” access to anellated triterpenoid lactams through a rearrangement reaction of platanic acid derived amides [45]. Thereby, the reaction of a variety of 3-*O*-acetylated platanic acid amides with sodium hydroxide in MeOH led to the neat formation of lactams. In continuation, we were interested in whether these anellated platanic acid derived lactams could also be converted to the corresponding cyclic amines by reduction with lithiumaluminium hydride to access compounds being able to inhibit cholinesterases.

3-*O*-Acetyl-platanic acid (**1**) was activated with oxalyl chloride and reacted with isobutylamine, phenethylamine or benzylamine to yield amides **2**–**4** [45]. Rearrangement [45] with NaOH/MeOH afforded lactams **5**–**7** whose reaction with $LiAlH_4$ gave products **8**–**10**. MS spectroscopic studies of **8**–**10** in the negative mode showed signals at m/z = 35 and m/z = 37 (^{35}Cl:^{37}Cl = 3:1)–thus corresponding to the presence of chloride ion. In the positive detection mode, on the other hand, of **8** m/z = 482 (corresponding to $[M\text{-}Cl]^+$) was observed. In the ^1H NMR spectrum of **8**, the signal for the C=CH_2 group being present in the starting material was no longer detectable, but a new signal at δ = 2.90 ppm was found. The ^{13}C NMR spectrum of this compound showed an additional signal at δ = 190 ppm, and in the ATR-IR spectrum a band was detected at ν = 1668 cm^{-1}; this signal corresponds to the presence of a C=N stretching vibration. From these results, we deduced that not a cyclic amine but the corresponding iminium salt **8** was formed. However, despite many attempts, no suitable crystals could be obtained for single-crystal X-ray analysis to elucidate

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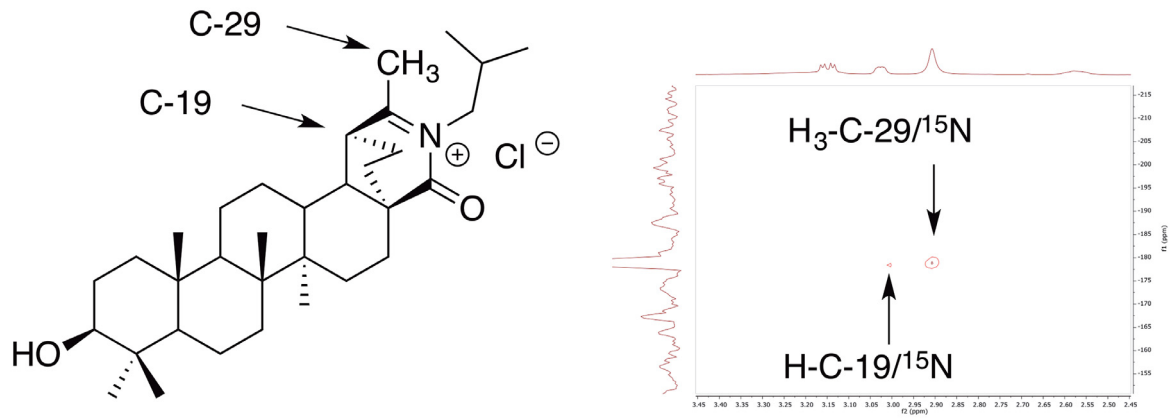
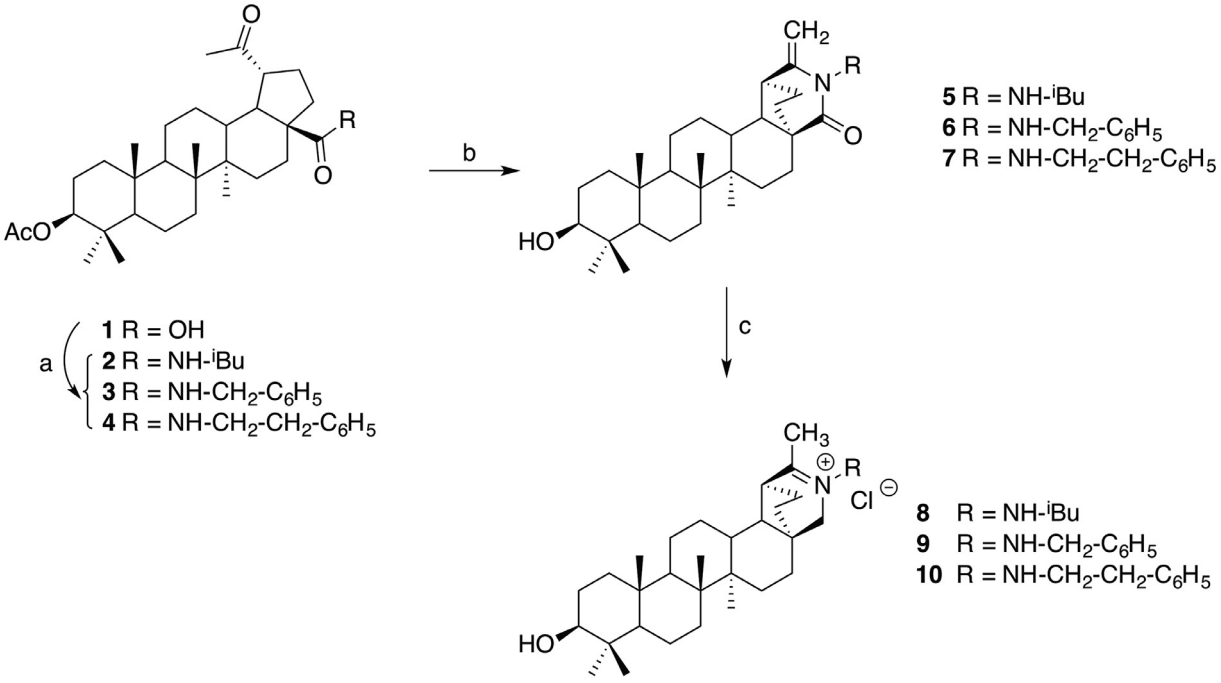


Fig. 1. gHMBC NMR ($^{15}N/^{1}H$) of **8**.



Scheme 1. Reactions and conditions: $(COCl)_2$, DMF (cat.), DCM, then R-NH_2, 2 h, 25 °C, 84% (of **2**), 88% (of **3**), and 91% (of **4**), respectively.

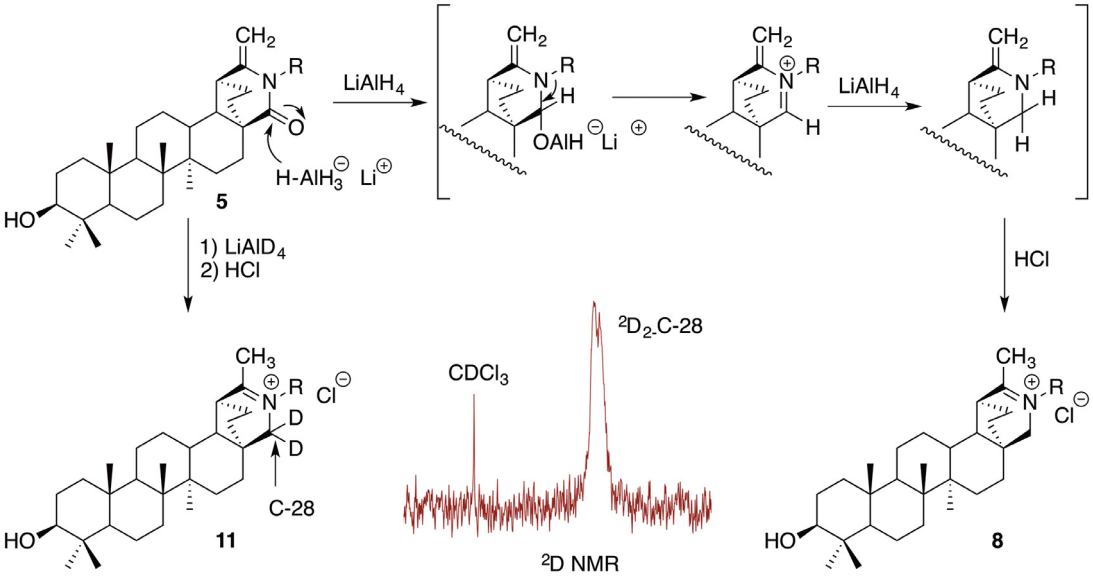
the structure in an unambiguous manner. Therefore, several extra NMR experiments were performed.

By means of gHMBCAD experiment, a ^{15}N chemical shift at $\delta = -179$ ppm could be obtained for **8**; this value is also in the expected range for iminium ions. In addition, coupling of the nitrogen to 29-H and 19-H was detected (Scheme 1 Fig. 1).

Extra gHSQCADTOCSY spectra confirmed these assignments and revealed the presence of additional couplings between 19-H with 13-H, 18-H, 21-H and 22-H thus further evidencing the postulated structure. Measurements at different temperatures and using different solvents/concentrations were made attempting to determine possible equilibria; no other structures were detected in these experiments.

The reduction of **5** with $LiAlD_4$ in deuterated solvent (Scheme 2) gave a corresponding analogous product **11**, whose ^{2}H NMR spectrum showed an incorporation of deuterium took place, and the carbonyl group present in the parent compound was reduced, while no further deuterium incorporation was observed. From these findings, a putative mechanism can be deduced: reduction of the amide occurs first with a subsequent reaction of the endo-cyclic iminium salt with $LiAlH_4$ to form the corresponding amine. Finally, a proton-catalyzed rearrangement (during acidic work-up) results in the formation the methyl-substituted iminium salt **5.**

In addition, several more NMR experiments were performed employing **8** such as an ASAP-HMQC NMR experiment [46–48]. Thereby, the ASAP (Acceleration by Sharing Adjacent Polarization) method belongs to a series of fast pulse techniques. As an advantage of these experiments the relaxation time between the individual scans during signal accumulation can be bypassed. As a consequence, the over-all acquisition time is less than one tenth of the usual measurement time–usually even less than one minute. gHSQC and gHMQC NMR spectra also contain in principle the same information as an ASAP-HMQC spectrum but the latter spectra can be obtained in a very short time and provide a sufficient resolution for initial investigations. Thus, this spectral technique represents a very useful experiment for time-saving initial structure elucidations also allowing a high sample throughput. Furthermore, they facilitate NMR investigations also for molecules of higher complexity such as a triterpene.



Scheme 2. Putative mechanism for the formation of **8** from **5** (R = ^{i}Bu); reduction of **5** with $LiAlD_4$ led to **11** holding two deuterium substituents at C-28. The deuterium NMR spectrum of **11** is depicted.

Table 1
Inhibition of AChE (from *electrophorus electricus*) and of BChE (from *equine serum*) as determined in Ellmanś assays; inhibition constants K_i and $K_{i'}$ are reported in μM and **GH** (galantamine hydrobromide) was used as a standard; the results are mean values resulting from triplicate experiments; % inhibition was determined at concentration of 10 μM.

	AChE K_i [μM] (% inhibition)	$K_{i'}$[μM]	type of inhibition	BChE K_i [μM] (% inhibition)	$K_{i'}$[μM]	Type of inhibition
GH	0.54 ± 0.01		competitive	9.37 ± 0.67		competitive
8	(<5)			0.38 ± 0.05 (95.6 ± 0.9)	1.48 ± 0.18	mixed
9	(42.1 ± 1.3)		–	3.06 ± 0.18 (82.3 ± 1.7)	7.02 ± 0.92	mixed
10	(<5)		–	0.80 ± 0.13 (95.4 ± 0.8)	2.87 ± 0.41	mixed

The formation of an iminium salt from a lactam, however, is not limited to alkyl-substituted lactams. Reduction of benzy-l or phenethyl- substituted lactams **6** or **7** also gave the iminium salts **9** and **10**, respectively. Even under "forced conditions" (microwave assisted synthesis, prolonged reaction time, reflux) the iminium salts could not be reduced to yield the corresponding amines. These iminium salts did not equilibrate to the corresponding amines; initial molecular modeling calculations also show that they are significantly more stable than the amines.

Compounds **8**–**10** were investigated for their ability to act as inhibitors of the enzymes acetyl- and butyrylcholinesterase (AChE, BChE). In Ellman assays, compounds **8** and **10** showed no inhibition on eeAChE, and **9** was only moderately active (Table 1), while all three compounds proved to be very good inhibitors of BChE. They are mixed-type inhibitors with **8** as the best compound showing very low K = 0.38 μM and Ki' = 1.48 μM, respectively. Thus, this compound is about as good an inhibitor as galantamine.

Stable triterpenoid iminium salts **8**–**10** are first in their class, and even more surprisingly compound **10** is an excellent inhibitor for the enzyme butyrylcholinesterase. The number of hitherto known iminium derived cholinesterase inhibitors is rather low [49]. Ongoing studies aim to reveal the biological potential of these novel compounds.

3. Conclusion

3-*O*-Acetyl-platanic acid was used as a easily accessible starting material for the synthesis of amides; these compounds hold a 30-nor-lupane skeleton but were converted in two steps into 21-aza-17,19-ethano-19-nor-urs-20-en-21-ium chlorides holding an ursane skeleton. These iminium salts are first in their class, proved quite stable, and some of them were shown as selective inhibitors of the enzyme butyrylcholinesterase. These compounds, however, were not able to inhibit the enzyme acetylcholinesterase.

4. Experimental

4.1. General

NMR spectra were recorded using the Agilent spectrometers DD2 500 MHz and VNMRS 400 MHz (δ given in ppm, J in Hz; typical experiments: APT, H–H-COSY, HMBC, HSQC, NOESY; calibration to the signal of the deuterated solvent, for ^{15}N measurements CH_3NO_2 was used for calibration), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument or on an Advion Expression CMS instrument. TLC was performed on silica gel (Macherey-Nagel, detection with cerium molybdate reagent); melting points are uncorrected (Leica hot stage microscope, or BUCHI melting point M-565), and elemental analyses were performed on a Foss-Heraeus Vario EL (CHNS) unit. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000 or on a Perkin-Elmer Spectrum Two (UATR Two Unit). A JASCO P-2000 polarimeter was used for the determination of the optical rotations. The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >96%. Column chromatography was performed on a Büchi Reveleris Prep purification system using Chromabond Flash cartridges (SiOH, 40.63 μm) from Macherey-Nagel or Reveleris high

resolution cartridges from Büchi. Platanic acid was obtained from betulinines (Stříbrná Skalice, Czech Republic) in >95% purity and used as received.

4.2. Cholinesterase assay

A BMG Labtech Spectrostar Omega working in the slow kinetics mode and measuring the absorbance at a distinct wavelength of λ = 412 nm with center scanning was used for the enzymatic studies. In short: A mixture of a DTNB solution (125 µL, 3 mM in 50 mM Tris–HCl buffer, pH 8), enzyme solution (25 µL, 2 U/mL) and compounds solutions (25 µL, 3 different concentrations and water as a blank) was incubated at 30 °C for 20 min. The substrate (25 µL, [ATChI] = 0.9375 mM, 0.625 mM, 0.325 mM, 0.1875 mM) was added to start the enzymatic reaction. The absorbance data was recorded under a controlled temperature of 30 °C for 30 min at 1 min intervals at λ=412 nm. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges. The used substrate concentration was 0.625 mM.

4.3. Syntheses

4.3.1. 3β-Acetyloxy-20-oxo-30-norlupan-28-oic acid (**1**)

Acetylation of platanic acid (5.13 g, 11.19 mmol) with acetic anhydride (3.2 mL, 33.50 mmol), in the presence of NEt_3 (4.5 mL, 32.30 mmol) and DMAP (cat.) in dry DCM (150 mL) for 2 days followed by usual aqueous work-up, gave compound **1** in 90% yield as colorless solid; yield: m.p. 255–257 °C (lit.: [50] 252–255 °C); $[\alpha]_D^{20}$ = −9.3° (*c* 0.50, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = −9.5° (*c* 0.80, $CHCl_3$)); R_f = 0.51 (silica gel, toluene/EtOAc/heptane/HCOOH, 80:26:10:5); MS (ESI, MeOH): *m/z* 999.1 (100%, $[2M\text{-}H]^-$).

4.3.2. 3β-Acetyloxy-N-(isobutyl)–20-oxo-30-norlupan-28-amide (**2**)

Following the procedure given for the synthesis of **3**, from **1** (2.0 g, 4.0 mmol), oxalyl chloride (1.0 mL, 12.0 mmol) and isobutylamine (1.32 mL, 13.0 mmol) followed by column chromatography (silica gel, hexanes/EtOAc, EtOAc: 20% → 30%) **2** (1.86 mg, 84%) was obtained as a colorless solid; m.p. 260–262 °C (lit.: [45] m.p. 259–261 °C (decomp.); R_f = 0.60 (silica gel, hexanes/EtOAc, 6:4); $[\alpha]_D^{20}$= −15.0° (*c* 0.40, $CHCl_3$) [lit.: [45] $[\alpha]_D^{20}$= −15.3° (*c* 0.326, $CHCl_3$)]; MS (ESI, MeOH): *m/z* 555.0 (100%, $[M\text{-}H]^-$).

4.3.3. 3β-Acetyloxy-N-benzyl-20-oxo-30-norlupan-28-amide (**3**)

Reaction of **1** (1.6, 3.2 mmol) in dry DCM (50 mL) with oxalyl chloride (1.2 mL, 14.0 mmol) and dry DMF (3 drops) at 0 °C (30 min) and 25 °C (60 min), removal of the volatiles, addition of dry DCM (40 mL), benzylamine (0.70 mL, 6.4 mmol) and NEt_3 (5 drops) in dry DCM (10 mL) and stirring for 120 min at 25 °C gave crude **3**. After usual aqueous work-up, and column chromatography (silica gel, EtOAc/hexanes, 3:1) compound **3** [(1.66 g, 88%) was obtained as a colorless solid. m.p. 290–293 °C (decomp.) (lit.: [45] 291–294 °C); $[\alpha]_D^{20}$ = +2.5° (*c* 0.40, $CHCl_3$) [lit.: [45] $[\alpha]_D^{20}$ = +2.2° (*c* 0.32, $CHCl_3$)]; R_f = 0.50 (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); MS (ESI, MeOH): *m/z* = 590.3 (100%, $[M + H]^+$), 612.3 (25%, $[M+Na]^+$).

4.3.4. 3β-Acetyloxy-N-phenethyl-20-oxo-30-norlupan-28-amide (**4**)

Following the procedure given for the synthesis of **3**, from **1** (1.4.0 g, 2.8 mmol), oxalyl chloride (0.7 mL, 8.4 mmol) and phenethylamine (1.16 mL, 9.2 mmol) followed by column chromatography (silica gel, hexanes/EtOAc: 30% → 40%) **4** (1.54 g, 91%) was obtained as a colorless solid; m.p. 222–226 °C (lit.: [45] 221–226 °C (decomp.); R_f = 0.65 (silica gel, hexanes/EtOAc, 6:4);; $[\alpha]_D^{20}$= −8.1° (*c* 0.35, $CHCl_3$) [lit.: [45] $[\alpha]_D^{20}$= −7.9° (*c* 0.30, $CHCl_3$)]; MS (ESI, MeOH): *m/z* 638.0 (100%, $[M+Cl]^-$), 602.3 (35%, $[M\text{-}H]^-$).

4.3.5. (3β) 21-Aza-17,19-ethano-3–hydroxy-21-isobutyl-20-methylidene-19,20-dinor-ursan-22-one (**5**)

Following the procedure given for the synthesis of **6** from **3** (550 mg, 1.0 mmol) followed by column chromatography (silica gel, hexanes/EtOAc, EtOAc: 10% → 20%) **5** (440 mg, 83%) was obtained as a colorless solid; m.p. 255–257 °C (lit.: [45] m.p. 257 °C (decomp.); R_f = 0.61 (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); $[\alpha]_D^{20}$ = +80.0° (*c* 0.32, $CHCl_3$) [lit.: [45] $[\alpha]_D^{20}$ = +81.8° (*c* 0.155, $CHCl_3$)]; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* 550.2 (100%, $[M + MeOH+Na]^+$), 496.0 (80%, $[M + H]^+$), 534.3 (60%, $[M + K]^+$).

4.3.6. (3β) 21-Aza-21-benzyl-17,19-ethano-3–hydroxy-20-methylidene-19,20-dinor-ursan-22-one (**6**)

To a solution of **3** (440 mg, 0.74 mmol) in MeOH (200 mL), powdered NaOH (20.0 g) was added, and the mixture was stirred for 1 week at 23 °C. The mixture was carefully neutralized by adding aqueous hydrochloric acid (20%); addition of cold water precipitated the product. The crude product was filtered off, dried and purified by column chromatography (silica gel, $CHCl_3$/acetone/hexanes, 95:05:20); yield: 410 mg (96%) as a colorless solid; m.p. 220–223 °C (lit.: [45] m.p. 222–224 °C; $[\alpha]_D^{20}$ = +76.3° (*c* 0.50, $CHCl_3$) [lit.:[45] $[\alpha]_D^{20}$ = +75.9° (*c* 0.30, $CHCl_3$); R_f = 0.60 (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); MS (ESI, MeOH): *m/z* = 530.5 (85%, $[M + H]^+$), 552.5 (15%, $[M+Na]^+$), 1081.6 (100%, $[2M+Na]^+$).

4.3.7. (3β) 21-Aza-17,19-ethano-3–hydroxy-20-methylidene-19,20-dinor-21-phenethyl-ursan-22-one (**7**)

Following the procedure given for the synthesis of **6** from **3** (450 mg, 0.72 mmol) followed by column chromatography (silica gel, hexanes/EtOAc, EtOAc: 20% → 30%) **7** (400 mg, 96%) was obtained as a colorless solid; m.p. 246–250 °C (lit.: [45] m.p. 250 °C (decomp.); R_f = 0.45 (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); $[\alpha]_D^{20}$ + 94.1° (*c* 0.20, $CHCl_3$) [lit.: [45] $[\alpha]_D^{20}$ + 95.5° (*c* 0.179, $CHCl_3$)]; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* 566.0 (100%, $[M + Na]^+$), 544.2 (20%, $[M + H]^+$).

4.4. General procedure for the synthesis of the iminium salts **8**–**10** (GPA)

To a solution of lactam **5**–**7** (1.0 equiv.) in dry THF (50 mL), $LiAlH_4$ (3.0 equiv.) was added, and the mixture was stirred for 72 h under reflux. After usual aqueous work up followed by column chromatography (silica gel, $CHCl_3$/MeOH 95:5) the respective product **8**–**10** was obtained each as a colorless solid.

4.4.1. (3β) 21-Aza-17,19-ethano-3–hydroxy-21-isobutyl-19-nor-urs-20-en-21-ium chloride (**8**)

According to GPA from **5** (300 mg, 0.606 mmol) followed by column chromatography (silica gel, $CHCl_3$/MeOH: 0% → 10%) **8** (158 mg, 54%) was obtained as a colorless solid; m.p. 208–212 °C (decomp.); R_f = 0.05 (silica gel, $CHCl_3$/MeOH, 95:5); $[\alpha]_D^{20}$ = +39.6° (*c* 0.152, $CHCl_3$) IR (ATR): ν = 3305 *w*, 2937 *m*, 1667 *w*, 1453 *w*, 1390 *w*, 1269 *w*, 1106 *w*, 1048 *w*, 984 *w*, 744 *s*, 657 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.09 (*dd*, *J* = 10.0 Hz, 1H, 30-Ha), 4.00–3.86 (*m*, 2H, 28-H_a, 30-H_b), 3.71 (*d*, *J* = 15.5 Hz, 1H, 28-H_b), 3.30 (*s*, 1H, OH), 3.19 (*dd*, *J* = 11.3, 4.7 Hz, 1H, 3-H), 3.01 (*dd*, *J* = 7.2, 3.3 Hz, 1H, 19-H), 2.73 (*s*, 3H, 29-H), 2.52 (*ddd*, *J* = 14.0, 9.2, 5.0 Hz, 1H, 21-H_a), 2.26–2.10 (*m*, 2H, 22-H_a, 31-H), 2.03 (*m*, 1H, 21-H_b), 1.69–1.14 (*m*, 19H, 1-H_a, 22-H_b, 15-H_a, 18-H, 2-H_a, 12-H_a, 15-H_b, 2-H_b, 6-H_a, 11-H_a, 13-H, 7-H, 6-H_b, 16-H_a, 11-H_b, 9-H, 16-H_b, 12-H_b), 1.07 (*d*, *J* = 6.6 Hz, 3H, 32-H), 0.99 (*d*, *J* = 6.6 Hz, 3H, 32-H), 0.97 (*s*, 3H, 27-H), 0.95 (*s*, 6H, 24-H, 26-H), 0.93–0.86 (*m*, 1H, 1-H_b), 0.81 (*s*, 3H, 25-H), 0.74 (*s*, 3H, 23-H), 0.70–0.65 (*m*, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 191.3 (C-20), 78.9 (C-3), 63.5 (C-30), 61.0 (C-28), 55.3 (C-5), 50.2 (C-9),

46.2 (C-19), 45.6 (C-18), 42.0 (C-8), 40.9 (C-14), 40.9 (C-17), 39.0 (C-4), 38.9 (C-22), 38.6 (C-1), 37.3 (C-10), 34.8 (C-13), 34.1 (C-7), 31.7 (C-21), 29.5 (C-15), 28.1 (C-24), 27.4 (C-2), 27.3 (31), 27.0 (C-12), 26.9 (C-16), 25.5 (C-29), 20.6 (C-11), 20.4 (C-32), 18.3 (C-6), 16.3 (C-25), 16.0 (C-27), 15.5 (C-23), 13.6 (C-26) ppm; ^{15}N NMR (51 MHz, $CDCl_3$): δ = 181.1 ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* 482.1 (100%, $[M\text{-}Cl]^+$); analysis calcd for $C_{33}H_{56}NO_3Cl$ (518.27): C 76.48, H 10.89, N 2.70; found: C 76.21, H 11.04, N 2.59.

*4.4.2. (3β) 21-Aza-21-benzyl-17,19-ethano-3–hydroxy-19-nor-urs-20-en-21-ium chloride (**9**)*

According to GPA from **6** (500 mg, 0.945 mmol) followed by column chromatography (silica gel, $CHCl_3$/MeOH: 0% → 10%) **9** (368 mg, 76%) was obtained as a colorless solid; m.p. 207–210 °C (decomp.); R_f = 0.05 (silica gel, $CHCl_3$/MeOH, 95:5); $[\alpha]_D^{20}$ = +76.5° (*c* 0.14, $CHCl_3$); IR (ATR): ν = 3351 *m*, 2939*s*, 1667 *m*, 1454 *m*, 1378 *m*, 1269 *w*, 1106 *w*, 1071 *w*, 1046 *m*, 985 *w*, 923 *w*, 729 *s*, 699 *s*, 621 *w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.28 (*m*, 5H, 32-H, 33-H, 34-H, 35-H, 36-H), 5.58 (*d*, *J* = 14.0 Hz, 1H, 28-H_a), 5.20 (*d*, *J* = 14.0 Hz, 1H, 28-H_b), 3.86–3.71 (*m*, 2H, 30-H), 3.15 (*dd*, *J* = 11.3, 4.7 Hz, 1H, 3-H), 3.03 (*dd*, *J* = 7.3, 3.4 Hz, 1H, 19-H), 2.91 (*s*, 3H, 29-H), 2.67–2.53 (*m*, 1H, 21-H_a), 2.22 (*s*, 1H, 22-H_a), 2.09–1.96 (*m*, 1H, 21-H_b), 1.68–0.99 (*m*, 19H, 1-H_a, 15-H_a, 12-H_a, 22-H_b, 18-H, 2-H, 6-H_a, 11-H, 12-H_b, 15-H_b, 13-H, 7-H, 6-H_b, 11-H_b, 16-H_a, 9-H, 16-H_b), 0.92 (*s*, 3H, 24-H), 0.87 (*s*, 3H, 26-H), 0.85–0.80 (*m*, 1H, 1-H_b), 0.77 (*s*, 3H, 25-H), 0.72 (*s*, 3H, 23-H), 0.65–0.57 (*m*, 1H, 5-H), 0.43 (*s*, 3H, 27-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 191.0 (C-20), 131.9 (C-31), 129.7 (C-32, C-36), 129.6 (C-34), 128.7 (C-33, C-35), 78.8 (C-3), 60.2 (C-30), 60.1 (C-28), 55.3 (C-5), 50.2 (C-9), 46.2 (C-19), 45.9 (C-18), 41.8 (C-8), 40.9 (C-14), 40.6 (C-17), 38.9 (C-4), 38.9 (C-1), 38.6 (C-22), 37.2 (C-10), 34.5 (C-13), 34.1 (C-7), 31.5 (C-21), 29.4 (C-15), 28.1 (C-24), 27.4 (C-2), 27.0 (C-16), 26.7 (C-12), 25.7 (C-29), 20.6 (C-11), 18.2 (C-6), 16.2 (C-25), 15.6 (C-27), 15.5 (C-23), 13.5 (C-26) ppm; ^{15}N NMR (51 MHz, $CDCl_3$): δ = 178.8 ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* 516.0 (100%, $[M\text{-}Cl]^+$); analysis calcd for $C_{36}H_{54}NOCl$ (552.27): C 78.29, H 9.76, N 2.54; found: C 77.97, H 10.03, N 2.39.

*4.4.3. (3β) 21-Aza-17,19-ethano-21-ethylphenyl-3–hydroxy-19-nor-urs-20-en-21-ium chloride (**10**)*

According to GPA from **7** (600 mg, 1.105 mmol) followed by column chromatography (silica gel, $CHCl_3$/MeOH: 0% → 10%) **10** (497 mg, 85%) was obtained as a colorless solid; m.p. 200–204 °C (decomp.); R_f = 0.09 (silica gel, $CHCl_3$/MeOH, 95:5);$[\alpha]_D^{20}$ = +33.1° (*c* 0.146, $CHCl_3$); IR (ATR): ν = 3315 *m*, 2939*s*, 1668 *m*, 1454*s*, 1377 *m*, 1048 *m*, 747 *s*, 701 *s* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.27 (*m*, 5H, 33-H, 34-H, 35-H, 36-H, 37-H), 4.62 (*dt*, *J* = 13.5, 6.4 Hz, 1H, 30-H_a), 4.34 (*dt*, *J* = 14.3, 7.3 Hz, 1H, 30-H_b), 3.93 (*d*, *J* = 15.8 Hz, 1H, 28-H_a), 3.62 (*d*, *J* = 15.9 Hz, 1H, 28-H_b), 3.29–3.12 (*m*, 2H, 3-H, 31-H_a), 3.01 (*dt*, *J* = 14.2, 7.3 Hz, 1H, 31-H_b), 2.86 (*dd*, *J* = 6.8, 2.9 Hz, 1H, 19-H), 2.35 (*s*, 3H, 29-H), 2.25–2.08 (*m*, 1H, 21-H_a), 2.04–1.90 (*m*, 2H, 21-H_b, 22-H_a), 1.72–1.12 (*m*, 19H, 1-H_a, 15-H_a, 12-H_a, 22-H_b, 18-H, 2-H, 6-H_a, 11-H, 12-H_b, 15-H_b, 13-H, 7-H, 6-H_b, 11-H_b, 16-H_a, 9-H, 16-H_b), 1.04 (*s*, 3H, 25-H), 0.95 (*s*, 3H, 24-H), 0.93 (*s*, 3H, 26-H), 0.92–0.87 (*m*, 1H, 1-H_b), 0.83 (*s*, 3H, 27-H), 0.74 (*s*, 3H, 23-H), 0.70–0.63 (*m*, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.0 (C-20), 136.2 (C-32), 129.3 (C-33, C-37), 129.1 (C-34, C-36), 127.9 (C-35), 78.9 (C-3), 61.1 (C-28), 58.2 (C-30), 55.4 (C-5), 50.2 (C-9), 46.0 (C-19), 45.6 (C-18), 42.0 (C-8), 40.9 (C-14), 40.8 (C-17), 38.9 (C-1), 38.9 (C-4), 38.2 (C-22), 37.3 (C-10), 34.6 (C-13), 34.2 (C-7), 33.6 (C-31), 32.1 (C-21), 29.4 (C-15), 28.1 (C-24), 27.4 (C-2), 26.9 (C-16), 26.9 (C-12), 24.8 (C-29), 20.4 (C-11), 18.3 (C-6), 16.5 (C-25), 16.3 (C-27), 15.5 (C-23), 13.6 (C-26) ppm; ^{15}N NMR (51 MHz, $CDCl_3$): δ = 182.5 ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* 530.2 (100%, $[M\text{-}Cl]^+$); analysis calcd for $C_{37}H_{56}NOCl$ (566.30): C 78.47, H 9.95, N 2.47; found: C 78.17, H 10.08, N 2.21.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Niels V. Heise: Investigation. **Dieter Ströhl:** Investigation. **Theresa Schmidt:** Investigation. **René Csuk:** Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Supplementary materials

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